

WHAT IS CLAIMED IS:

1 1. A modified oligonucleotide comprising at least two bases selected
2 from the group consisting of unsubstituted and 3-substituted pyrazolo[3,4-d]pyrimidine
3 bases.

1 2. A modified oligonucleotide of claim 1, further comprising a covalently
2 attached minor groove binder.

1 3. A modified oligonucleotide of claim 1, further comprising at least one
2 covalently attached reporter group.

1 4. A modified oligonucleotide of claim 1, further comprising at least one
2 covalently attached quencher.

1 5. A modified oligonucleotide of claim 3, wherein said reporter group is a
2 fluorophore.

1 6. A modified oligonucleotide of claim 3, wherein said reporter group is a
2 fluorophore and said modified oligonucleotide further comprises an attached quencher.

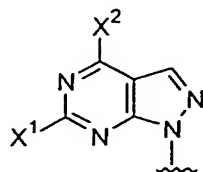
1 7. A modified oligonucleotide of claim 1, comprising from 4 to 70 bases.

1 8. A modified oligonucleotide of claim 1, comprising from 4 to 70 bases
2 and further comprising an attached minor groove binder.

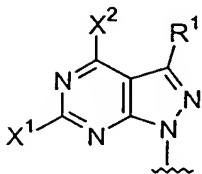
1 9. A modified oligonucleotide of claim 1, comprising from 4 to 70 bases
2 and further comprising an attached fluorophore and a quencher.

1 10. A modified oligonucleotide of claim 2, comprising from 4 to 70 bases
2 and further comprising an attached fluorophore and a quencher.

1 11. A modified oligonucleotide of claim 1, wherein at least one base of
2 said at least two bases is an unsubstituted pyrazolo[3,4-d]pyrimidine base having the formula:



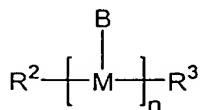
and at least one of said at least two bases is a 3-substituted pyrazolo[3,4-d]pyrimidine base having the formula:



wherein

each of said X^1 and X^2 groups is a member independently selected from the group consisting of H, OH, NH_2 and a protected amino group; and each of said R^1 groups is a member independently selected from the group consisting of $(\text{C}_1\text{-C}_{12})$ heteroalkyl, $(\text{C}_2\text{-C}_{12})$ heteroalkenyl, $(\text{C}_2\text{-C}_{12})$ heteroalkynyl, $-\text{O}-(\text{C}_1\text{-C}_{12})$ alkyl, $-\text{O}-(\text{C}_2\text{-C}_{12})$ alkenyl, $-\text{O}-(\text{C}_2\text{-C}_{12})$ alkynyl, $-\text{S}-(\text{C}_1\text{-C}_{12})$ alkyl, $-\text{S}-(\text{C}_2\text{-C}_{12})$ alkenyl, $-\text{S}-(\text{C}_2\text{-C}_{12})$ alkynyl, heterocyclyl $(\text{C}_1\text{-C}_{12})$ alkyl, heterocyclyl $(\text{C}_2\text{-C}_{12})$ alkenyl, heterocyclyl $(\text{C}_2\text{-C}_{12})$ alkynyl, aryl $(\text{C}_1\text{-C}_{12})$ alkyl, aryl $(\text{C}_2\text{-C}_{12})$ alkenyl, aryl $(\text{C}_2\text{-C}_{12})$ alkynyl, aryl, heterocyclyl, halogen, $-\text{CN}$, $-\text{CONH}_2$ and protected forms thereof.

12. A modified oligonucleotide of claim 1, having the formula:



R^2 represents a first end of said modified oligonucleotide;

R^3 represents a second end of said modified oligonucleotide;

the subscript n is an integer of from 4 to 70;

each B is a member independently selected from the group consisting of adenine, thymine, cytosine, guanine, uracil, a pyrazolo[3,4-d]pyrimidine and a 3-substituted pyrazolo[3,4-d]pyrimidine; and

each M is a member selected from the group consisting of an oligomer-forming sugar and a peptide-nucleic acid-forming amino acid.

13. A modified oligonucleotide of claim 12, wherein at least one M is a non-natural oligomer-forming sugar.

14. A modified oligonucleotide comprising at least one 5-substituted pyrimidine base and at least one unsubstituted or 3-substituted pyrazolo[3,4-d]pyrimidine base.

1 15. A modified oligonucleotide of claim 14, further comprising a
2 covalently attached minor groove binder.

1 16. A modified oligonucleotide of claim 14, further comprising at least one
2 covalently attached reporter group.

1 17. A modified oligonucleotide of claim 14, further comprising at least one
2 covalently attached quencher.

1 18. A modified oligonucleotide of claim 16, wherein said reporter group is
2 a fluorophore.

1 19. A modified oligonucleotide of claim 16, wherein said reporter group is
2 a fluorophore and said modified oligonucleotide further comprises an attached quencher.

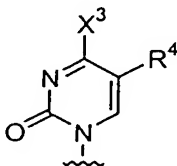
1 20. A modified oligonucleotide of claim 14, comprising from 4 to 70
2 bases.

1 21. A modified oligonucleotide of claim 14, comprising from 4 to 70 bases
2 and further comprising an attached minor groove binder.

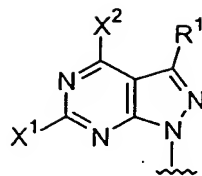
1 22. A modified oligonucleotide of claim 14, comprising from 4 to 70 bases
2 and further comprising an attached fluorophore and a quencher.

1 23. A modified oligonucleotide of claim 15, comprising from 4 to 70 bases
2 and further comprising an attached fluorophore and a quencher.

1 24. A modified oligonucleotide of claim 14, wherein said at least one 5-
2 substituted pyrimidine base having a formula selected from the group consisting of:



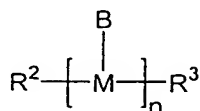
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4 and said at least one unsubstituted or 3-substituted pyrazolo[3,4-d]pyrimidine base selected
5 from the group consisting of:



wherein

each of said X¹, X² and X³ groups is a member independently selected from the group consisting of H, OH, NH₂ and a protected amino group; and each of said R¹ and R⁴ groups is a member independently selected from the group consisting of (C₁-C₁₂)heteroalkyl, (C₂-C₁₂)heteroalkenyl, (C₂-C₁₂)heteroalkynyl, -O-(C₁-C₁₂)alkyl, -O-(C₂-C₁₂)alkenyl, -O-(C₂-C₁₂)alkynyl, -S-(C₁-C₁₂)alkyl, -S-(C₂-C₁₂)alkenyl, -S-(C₂-C₁₂)alkynyl, heterocyclyl(C₁-C₁₂)alkyl, heterocyclyl(C₂-C₁₂)alkenyl, heterocyclyl(C₂-C₁₂)alkynyl, aryl(C₁-C₁₂)alkyl, aryl(C₂-C₁₂)alkenyl, aryl(C₂-C₁₂)alkynyl, aryl, heterocyclyl, halogen, -CN, -CONH₂ and protected forms thereof.

25. A modified oligonucleotide of claim 14, having the formula:



R² represents a first end of said modified oligonucleotide;

R³ represents a second end of said modified oligonucleotide;

the subscript n is an integer of from 4 to 70;

each B is a member independently selected from the group consisting of adenine, thymine, cytosine, guanine, uracil, a 5-substituted pyrimidine and a 3-substituted pyrazolo[3,4-d]pyrimidine; and

each M is a member selected from the group consisting of an oligomer-forming sugar and a peptide-nucleic acid-forming amino acid.

26. A modified oligonucleotide of claim 25, wherein at least one M is a locked oligomer-forming sugar.

27. A modified oligonucleotide comprising from about 4 to about 70 bases and an attached minor groove binder, wherein at least one of said bases is replaced by a modified base selected from the group consisting of 5-substituted pyrimidines and unsubstituted or 3-substituted pyrazolo[3,4-d]pyrimidines.

1 28. A modified oligonucleotide of claim 27, wherein at least one of said
2 bases is replaced by a 5-substituted pyrimidine.

1 29. A modified oligonucleotide of claim 27, wherein at least one of said
2 bases is replaced by a 3-substituted pyrazolo[3,4-d]pyrimidine.

1 30. A modified oligonucleotide of claim 27, further comprising an attached
2 reporter group.

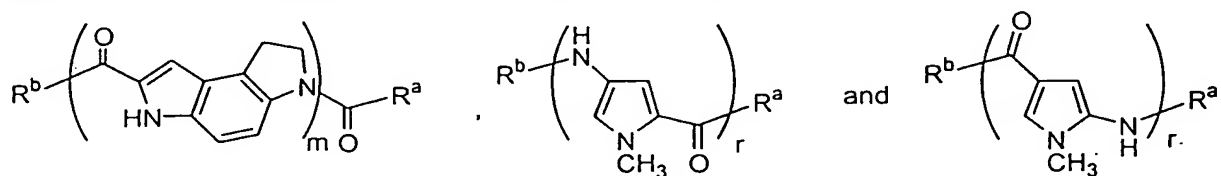
1 31. A modified oligonucleotide of claim 30, wherein said reporter group is
2 a fluorophore and said modified oligonucleotide further comprises an attached quencher.

1 32. A modified oligonucleotide of claim 31, comprising of from about 4 to
2 about 20 bases.

1 33. A modified oligonucleotide of claim 32, wherein said fluorophore is
2 attached at the 5'-terminus and said quencher is attached at the 3'-terminus.

1 34. A modified oligonucleotide of claim 32, wherein said fluorophore is
2 attached at the 3'-terminus and said quencher is attached at the 5'-terminus.

1 35. A modified oligonucleotide of claim 27, wherein said minor groove
2 binder has a formula selected from the group consisting of:



4 wherein

5 the subscript m is an integer of from 2 to 5;

6 the subscript r is an integer of from 2 to 10; and

7 each R^a and R^b is independently a linking group to said modified oligonucleotide, H,

8 $-OR^c$, $-NR^cR^d$, $-COOR^c$ and $-CONR^cR^d$ wherein each R^c and R^d is selected

9 from the group consisting of H, (C_1-C_{12}) heteroalkyl, (C_2-C_{12}) heteroalkenyl,

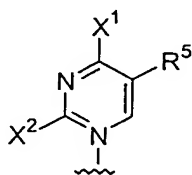
10 (C_2-C_{12}) heteroalkynyl, (C_1-C_{12}) alkyl, (C_2-C_{12}) alkenyl, (C_2-C_{12}) alkynyl,

11 aryl (C_1-C_{12}) alkyl and aryl.

1 36. A modified oligonucleotide of claim 30, wherein said reporter group is
2 selected from the group consisting of a resorufin dye, a coumarin dye, a rhodamine dye, a
3 cyanine dye, a BODIPY dye, a fluorescein dye and a pyrene.

1 37. A modified oligonucleotide of claim 31, wherein said reporter group is
2 selected from the group consisting of resorufin dye, a coumarin dye, a rhodamine dye, a
3 cyanine dye, a BODIPY dye, a fluorescein dye and a pyrene, and said quencher is selected
4 from the group consisting of non-fluorescent quenchers, 1-aza-1,2-diphenylethene derivatives
5 and rhodamine derivatives.

1 38. A modified oligonucleotide of claim 27, wherein said modified base is
2 selected from the group consisting of



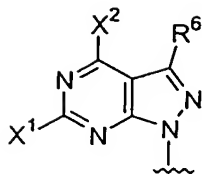
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4 wherein

5 X¹ and X² are each independently selected from the group consisting of H, NH₂, OH
6 and SH; and

7 R⁵ is a member selected from the group consisting of (C₁-C₁₂)heteroalkyl, (C₂-
8 C₁₂)heteroalkenyl, (C₂-C₁₂)heteroalkynyl, -O-(C₁-C₁₂)alkyl, -O-(C₂-
9 C₁₂)alkenyl, -O-(C₂-C₁₂)alkynyl, -S-(C₁-C₁₂)alkyl, -S-(C₂-C₁₂)alkenyl, -S-(C₂-
10 C₁₂)alkynyl, heterocyclyl(C₁-C₁₂)alkyl, heterocyclyl(C₂-C₁₂)alkenyl,
11 heterocyclyl(C₂-C₁₂)alkynyl, aryl(C₁-C₁₂)alkyl, aryl(C₂-C₁₂)alkenyl, aryl(C₂-
12 C₁₂)alkynyl, aryl, heterocyclyl, halogen, -CN, -CONH₂ and protected forms
13 thereof.

1 39. A modified oligonucleotide of claim 38, wherein said heterocyclyl and
2 aryl groups are selected from the group consisting of phenyl, tolyl, pyridyl, thiazolyl,
3 imidazolyl, furanyl, oxazolyl, thienyl, pyrrolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl,
4 indolyl, triazinyl, pyrimidinyl and naphthyl.

1 40. A modified oligonucleotide of claim 27, wherein said modified base is
2 selected from the group consisting of

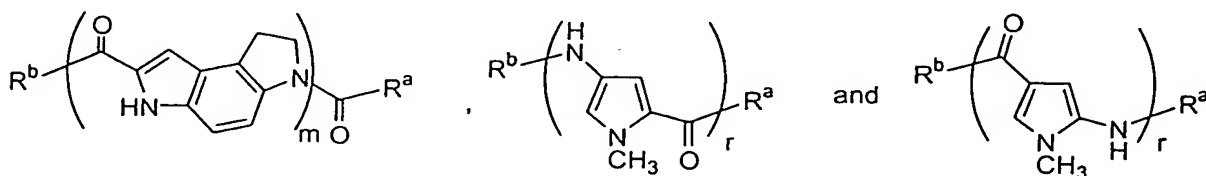


wherein

each of said X^1 and X^2 groups is a member independently selected from the group consisting of H, OH, NH_2 and a protected amino group; and R^6 is a member selected from the group consisting of (C_1-C_{12}) heteroalkyl, (C_2-C_{12}) heteroalkenyl, (C_2-C_{12}) heteroalkynyl, $-O-(C_1-C_{12})$ alkyl, $-O-(C_2-C_{12})$ alkenyl, $-O-(C_2-C_{12})$ alkynyl, $-S-(C_1-C_{12})$ alkyl, $-S-(C_2-C_{12})$ alkenyl, $-S-(C_2-C_{12})$ alkynyl, heterocyclyl (C_1-C_{12}) alkyl, heterocyclyl (C_2-C_{12}) alkenyl, heterocyclyl (C_2-C_{12}) alkynyl, aryl (C_1-C_{12}) alkyl, aryl (C_2-C_{12}) alkenyl, aryl (C_2-C_{12}) alkynyl, aryl, heterocyclyl, halogen, $-CN$, $-CONH_2$ and protected forms thereof.

41. A modified oligonucleotide of claim 40, wherein said heterocyclyl and aryl groups are selected from the group consisting of phenyl, tolyl, pyridyl, thiazolyl, imidazolyl, furanyl, oxazolyl, thienyl, pyrrolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, indolyl, triazinyl, pyrimidinyl and naphthyl.

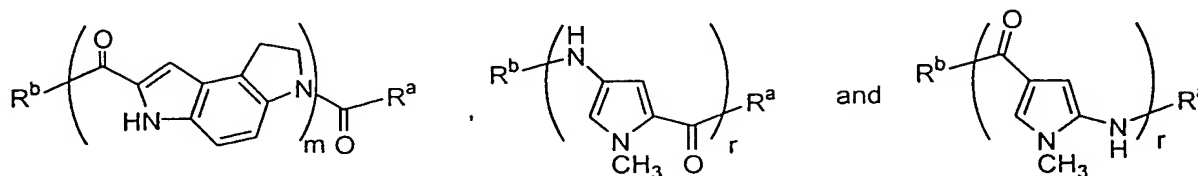
42. A modified oligonucleotide of claim 39, wherein said minor groove binder has a formula selected from the group consisting of:



wherein

the subscript m is an integer of from 2 to 5;
the subscript r is an integer of from 2 to 10; and
each R^a and R^b is independently a linking group to said modified oligonucleotide, H, $-OR^c$, $-NR^cR^d$, $-COOR^c$ and $-CONR^cR^d$ wherein each R^c and R^d is selected from the group consisting of H, (C_1-C_{12}) heteroalkyl, (C_2-C_{12}) heteroalkenyl, (C_2-C_{12}) heteroalkynyl, (C_1-C_{12}) alkyl, (C_2-C_{12}) alkenyl, (C_2-C_{12}) alkynyl, aryl (C_1-C_{12}) alkyl and aryl.

43. A modified oligonucleotide of claim 41, wherein said minor groove binder has a formula selected from the group consisting of:



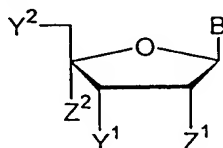
wherein

the subscript n is an integer of from 2 to 5;

the subscript r is an integer of from 2 to 10; and

each R^a and R^b is independently a linking group to said modified oligonucleotide, H, $-OR^c$, $-NR^cR^d$, $-COOR^c$ and $-CONR^cR^d$ wherein each R^c and R^d is selected from the group consisting of H, (C_1-C_{12}) heteroalkyl, (C_2-C_{12}) heteroalkenyl, (C_2-C_{12}) heteroalkynyl, (C_1-C_{12}) alkyl, (C_2-C_{12}) alkenyl, (C_2-C_{12}) alkynyl, aryl (C_1-C_{12}) alkyl and aryl.

44. A compound having the formula:



wherein

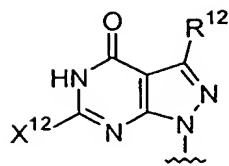
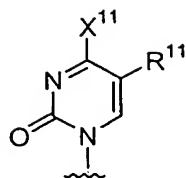
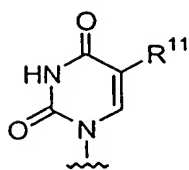
Z^1 is a member selected from the group consisting of H, F and OR^a wherein R^a is a member selected from the group consisting of H, (C_1-C_8) alkyl and a hydroxy protecting group;

Z^2 is a member selected from the group consisting of H and (C_1-C_8) alkyl, or optionally Z^2 is combined with Z^1 for form a five- to seven-membered ring;

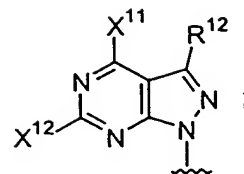
Y^1 is a member selected from the group consisting of OH, a protected hydroxy group and $O-P^1$, wherein P^1 is a phosphoramidite or H-phosphonate group;

Y^2 is a member selected from the group consisting of OH, a protected hydroxy group and $O-P^2$, wherein P^2 is a phosphoramidite, H-phosphonate, monophosphate, diphosphate or triphosphate; and

B is a modified nucleotide selected from the group consisting of:



and



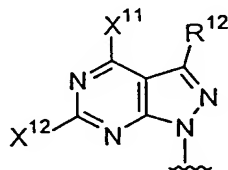
wherein

X^{11} and X^{12} are each independently selected from the group consisting of H, NH_2 and a protected amino group;

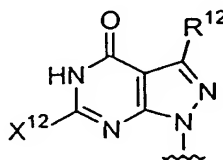
each R^{11} is independently selected from the group consisting of protected or unprotected forms of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-ynyl, 3-methoxyprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl; and

each R^{12} is independently selected from the group consisting of protected or unprotected forms of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-ynyl, 3-methoxyprop-1-ynyl, 4-hydroxy-1-butynyl, 3-(hydroxymethyl)-4-hydroxy-1-butynyl, heterocyclyl(C_1 - C_{12})alkyl, heterocyclyl(C_2 - C_{12})alkenyl, heterocyclyl(C_2 - C_{12})alkynyl and heterocyclyl, with the proviso that R^{12} is other than 2-pyridylethynyl.

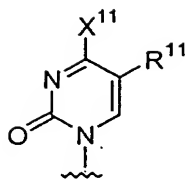
45. A compound of claim 44, wherein B is



46. A compound of claim 44, wherein B is



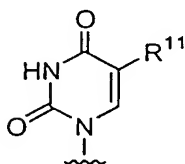
47. A compound of claim 44, wherein B is



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48. A compound of claim 44, wherein B is



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49. A compound of claim 45, wherein X¹¹ and X¹² are each NH₂.

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50. A compound of claim 49, wherein Y¹ is O-P¹, Y² is a protected hydroxy, Z¹ is H, R¹² is selected from the group consisting of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl.

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51. A compound of claim 50, wherein Y¹ is -O-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] and Y² is -O-(4,4'-dimethoxytrityl).

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52. A compound of claim 45, wherein X¹¹ is NH₂ and X¹² is H.

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53. A compound of claim 52, wherein Y¹ is O-P¹, Y² is a protected hydroxy, Z¹ is H, R¹² is selected from the group consisting of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl.

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54. A compound of claim 53, wherein Y¹ is -O-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] and Y² is -O-(4,4'-dimethoxytrityl).

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55. A compound of claim 46, wherein X¹² is H or NH₂.

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56. A compound of claim 55, wherein Y¹ is O-P¹, Y² is a protected hydroxy, Z¹ is H, R¹² is selected from the group consisting of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl.

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57. A compound of claim 56, wherein X¹ is

2 -O-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] and X^2 is -O-(4,4'-
3 dimethoxytrityl).

1 58. A compound of claim 47, wherein X^{11} is NH_2 .

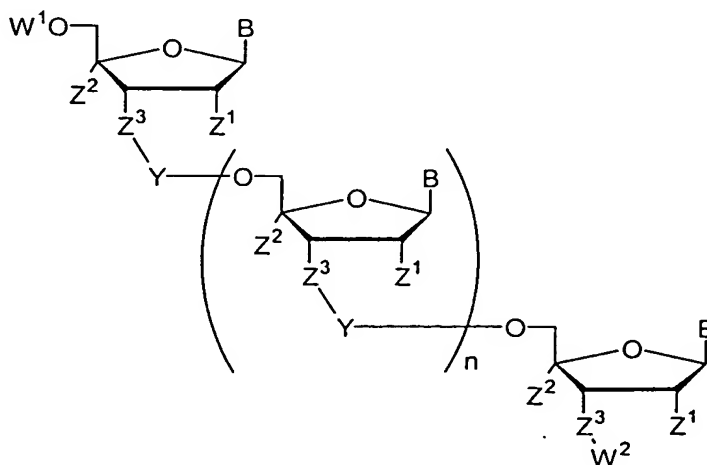
1 59. A compound of claim 58, wherein Y^1 is $O-P^1$, Y^2 is a protected
2 hydroxy, Z^1 is H, R^{11} is selected from the group consisting of 3-hydroxyprop-1-ynyl, 3-
3 aminoprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl.

1 60. A compound of claim 59, wherein Y^1 is
2 -O-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] and Y^2 is -O-(4,4'-
3 dimethoxytrityl).

1 61. A compound of claim 48, wherein Y^1 is $O-P^1$, Y^2 is a protected
2 hydroxy, Z^1 is H, and R^{11} is selected from the group consisting of 3-hydroxyprop-1-ynyl,
3 3-aminoprop-1-ynyl, 4-hydroxy-1-butynyl and 3-(hydroxymethyl)-4-hydroxy-1-butynyl.

1 62. A compound of claim 61, wherein Y^1 is
2 -O-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] and Y^2 is -O-(4,4'-
3 dimethoxytrityl).

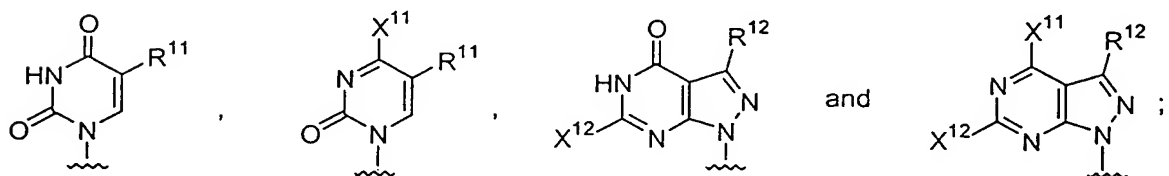
1 63. An oligonucleotide having the formula:



2 wherein

4 each Z^1 a member independently selected from the group consisting of H, F and
5 OR^a wherein R^a is a member selected from the group consisting of H, $(C_1-$
6 $C_8)$ alkyl and a hydroxy protecting group;

each Z^2 is a member selected from the group consisting of H and (C₁-C₈)alkyl, or
 optionally Z^2 and Z^1 on one or more of the same furanose rings are
 combined to form a five- to seven-membered ring;
 each Z^3 is selected from the group consisting of O, S and NH;
 each Y is a member independently selected from the group consisting of P(O)OH,
 P(S)OH and P(O)CH₃;
 the subscript n is an integer of from 1 to 98;
 W^1 and W^2 are each independently selected from the group consisting of H, a
 monophosphate, a diphosphate, a triphosphate and a minor groove binder-
 linking group moiety having an optionally attached reporter group; and
 each B is a member independently selected from the group consisting of adenine,
 guanine, cytosine, uridine and modified bases of the formula:



wherein

X^{11} and X^{12} are each independently selected from the group consisting of
 H, NH₂ and a protected amino group;

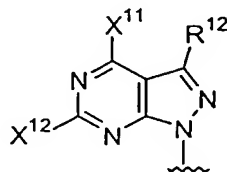
each R^{11} is independently selected from the group consisting of protected
 or unprotected forms of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-
 ynyl, 3-methoxyprop-1-ynyl, 4-hydroxy-1-butynyl and 3-
 (hydroxymethyl)-4-hydroxy-1-butynyl; and

each R^{12} is independently selected from the group consisting of protected
 or unprotected forms of 3-hydroxyprop-1-ynyl, 3-aminoprop-1-
 ynyl, 3-methoxyprop-1-ynyl, 4-hydroxy-1-butynyl, 3-
 (hydroxymethyl)-4-hydroxy-1-butynyl, heterocyclyl(C₁-C₁₂)alkyl,
 heterocyclyl(C₂-C₁₂)alkenyl, heterocyclyl(C₂-C₁₂)alkynyl and
 heterocyclyl, with the proviso that R^{12} is other than 2-
 pyridylethynyl; and

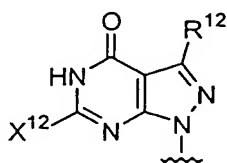
with the further proviso that at least one of said Bs is selected from said
 modified bases, and optionally, one or more of said Bs has an
 attached minor groove binder-linking group moiety, reporter group
 or a combination thereof.

1 64. An oligonucleotide of claim 63, wherein n is an integer of from 4
2 to 30.

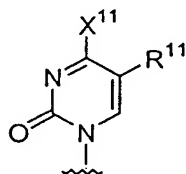
1 65. An oligonucleotide of claim 63, wherein at least one B is



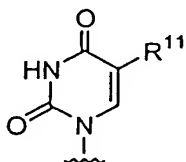
1 66. An oligonucleotide of claim 63, wherein at least one B is



1 67. An oligonucleotide of claim 63, wherein at least one B is



1 68. An oligonucleotide of claim 63, wherein at least one B is



1 69. An oligonucleotide of claim 63, wherein W¹ is a minor groove
2 binder-linking group moiety.

1 70. An oligonucleotide of claim 63, wherein W¹ is a minor groove
2 binder-linking group moiety having an attached reporter group.

1 71. An oligonucleotide of claim 63, wherein W² is a minor groove
2 binder-linking group moiety.

1 72. An oligonucleotide of claim 63, wherein W^2 is a minor groove
2 binder-linking group moiety having an attached reporter group.

1 73. An oligonucleotide of claim 63, wherein at least one Z^3 is NH.

1 74. A modified oligonucleotide array, said array comprising a solid
2 support and a plurality of attached oligonucleotides, wherein at least 50% of the
3 oligonucleotides in said array contain a modified base selected from the group consisting
4 of unsubstituted pyrazolo[3,4-d]pyrimidines, 3-substituted pyrazolo[3,4-d]pyrimidines
5 and 5-substituted pyrimidines.

1 75. A modified oligonucleotide array of claim 74, wherein said
2 attached oligonucleotides have T_m s within about 2°C of each other and basepair lengths
3 within about 2 bases of each other.

1 76. A modified oligonucleotide array of claim 74, wherein said
2 attached oligonucleotides have T_m s within about 1°C of each other and basepair lengths
3 within about 2 bases of each other.

1 77. A modified oligonucleotide array of claim 74, wherein said array
2 comprises from about 10 to about 10,000 attached oligonucleotides, each having T_m s
3 within about 2°C of each other and basepair lengths within about 2 bases of each other.

1 78. A modified oligonucleotide array of claim 74, wherein said array
2 comprises from about 10 to about 10,000 attached oligonucleotides, a portion of said
3 attached oligonucleotides having a covalently attached minor groove binder.

1 79. A composition comprising a plurality of modified oligonucleotides
2 having at least one base selected from the group consisting of unsubstituted pyrazolo[3,4-
3 d]pyrimidines, 3-substituted pyrazolo[3,4-d]pyrimidines and 5-substituted pyrimidines,
4 and further having an attached fluorophore.

1 80. A composition of claim 79, wherein each of said plurality of
2 oligonucleotides comprises an attached fluorophore and an attached quencher.

- 1 81. A composition of claim 80, wherein each of said modified
2 oligonucleotides are from 4 to 30 bases in length and have T_m s that are within about 2°C
3 of each other.
- 1 82. A composition of claim 81, wherein said plurality is from about 6
2 to about 100.
- 1 83. A method for distinguishing polynucleotides with related
2 sequences, the method comprising:
3 (a) contacting a modified oligonucleotide having a defined sequence
4 comprising at least one 3-substituted pyrazolo[3,4-*d*]pyrimidine or 5-substituted
5 pyrimidine in place of a purine or pyrimidine base with at least two polynucleotides,
6 wherein one of the polynucleotides has a target sequence that is perfectly complementary
7 to the modified oligonucleotide and at least one of the other polynucleotides has a target
8 sequence with at least one base mismatch; and
9 (b) determining the degree of hybridization between the modified
10 oligonucleotide and each of the polynucleotides.
- 1 84. A method in accordance with claim 83, wherein said modified
2 oligonucleotide further comprises a reporter group.
- 1 85. A method in accordance with claim 84, wherein said reporter group
2 is a fluorophore.
- 1 86. A method in accordance with claim 83, wherein said modified
2 oligonucleotide further comprises a minor groove binder.
- 1 87. A method in accordance with claim 83, wherein said modified
2 oligonucleotide further comprises a minor groove binder and a fluorophore.
- 1 88. A method in accordance with claim 83, wherein said modified
2 oligonucleotide further comprises a minor groove binder, a fluorophore and a quencher.
- 1 89. A method for detecting the presence of a target sequence in a
2 polynucleotide, the method comprising:

3 (a) incubating a polynucleotide to be tested for the presence of the target
4 sequence with a modified oligonucleotide having a sequence that is substantially
5 complementary to the target sequence under hybridization conditions; and
6 (b) identifying hybridized nucleic acids;
7 wherein said modified oligonucleotide comprises at least one 3-substituted
8 pyrazolo[3,4-*d*]pyrimidine in place of a purine residue.

1 90. A method in accordance with claim 89, wherein said incubating is
2 conducted in the presence of a cleavase enzyme.

1 91. A method in accordance with claim 89, wherein said modified
2 oligonucleotide further comprises a reporter group.

1 92. A method in accordance with claim 91, wherein said reporter group
2 is a fluorophore.

1 93. A method in accordance with claim 92, said modified
2 oligonucleotide further comprising an attached quencher.

1 94. A method in accordance with claim 89, wherein said modified
2 oligonucleotide further comprises an attached minor groove binder.

1 95. A method for primer extension, the method comprising incubating
2 a polynucleotide containing a target sequence with one or more oligonucleotide primers
3 complementary to the target sequence, in the presence of a polymerizing enzyme and
4 nucleotide substrates under conditions favorable for polymerization; wherein at least one
5 of the oligonucleotide primers contains a modified base selected from the group
6 consisting of an unsubstituted pyrazolo[3,4-*d*]pyrimidine, a 3-substituted pyrazolo[3,4-
7 *d*]pyrimidine and a 5-substituted pyrimidine base, in place of a purine or pyrimidine base.

1 96. A method in accordance with claim 95, wherein one of said
2 oligonucleotide primers is extended with a single base.

1 97. A method in accordance with claim 95, wherein said at least one of
2 said oligonucleotide primers further comprises an attached minor groove binder.

1 98. A method in accordance with claim 95, wherein said incubating is
2 part of an amplification reaction.

1 99. A method in accordance with claim 98, wherein said amplification
2 reaction is a polymerase chain reaction.

1 100. A method in accordance with claim 95, wherein said modified
2 oligonucleotide further comprises a covalently attached minor groove binder.

1 101. A method for determining the nucleotide sequence of a
2 polynucleotide, the method comprising:

3 (a) incubating the polynucleotide with a modified oligonucleotide array
4 under hybridization conditions; and

5 (b) determining to which of the modified oligonucleotides in the array the
6 polynucleotide hybridizes;

7 wherein a plurality of the modified oligonucleotides comprise at least one
8 3-substituted pyrazolo[3,4-*d*]pyrimidine in place of a purine base.

1 102. A method in accordance with claim 101, wherein said array
2 comprises from 10 to 100,000 different modified oligonucleotides.

1 103. A method in accordance with claim 101, wherein said array
2 comprises from 10 to 1000 different modified oligonucleotides.

1 104. A method for determining the nucleotide sequence of a target
2 sequence in a polynucleotide, the method comprising:

3 (a) contacting a polynucleotide comprising the target sequence with at
4 least two oligonucleotides of known sequence wherein one or more purine residues of the
5 oligonucleotides are replaced by a 3-substituted pyrazolo[3,4-*d*]pyrimidine, and wherein
6 one of the at least two oligonucleotides has a sequence that is perfectly complementary to
7 the target sequence and at least one other of the oligonucleotides has a related target
8 sequence and incubating each of the oligonucleotides with the polynucleotide under
9 hybridization conditions; and

10 (b) determining the degree of hybridization between each of the
11 oligonucleotides and the polynucleotide.

1 105. A method in accordance with claim 104, wherein at least one of
2 said modified oligonucleotides further comprises a reporter group.

1 106. A method in accordance with claim 104, wherein at least one of
2 said modified oligonucleotides further comprises a minor groove binder.

1 107. A method in accordance with claim 104, wherein at least one of
2 said modified oligonucleotides further comprises a minor groove binder and a reporter
3 group.

1 108. A method for examining gene expression in a cell, the method
2 comprising:

3 (a) incubating a population of polynucleotides representative of the genes
4 expressed in the cell with an oligonucleotide array comprising a plurality of modified
5 oligonucleotides of different sequences under hybridization conditions, and

6 (b) determining which of the modified oligonucleotides in the array
7 become hybridized to polynucleotides;

8 wherein said modified oligonucleotides comprise at least one 3-substituted
9 pyrazolo[3,4-*d*]pyrimidine in place of a purine.

1 109. A method for identifying a mutation in a target sequence of a gene
2 of interest, the method comprising:

3 (a) incubating a polynucleotide comprising the target sequence with an
4 array of oligonucleotides of different sequences, wherein the different sequences include
5 the wild-type target sequence and different mutant target sequences, under hybridization
6 conditions; and

7 (b) determining which of the oligonucleotides in the array become
8 hybridized to the polynucleotide;

9 wherein one or more purine residues in each of the oligonucleotides are
10 replaced with a 3-substituted pyrazolo[3,4-*d*]pyrimidine.